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Comparison of glucose concentration stability in serum and plasma tubes

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Abstract

Aim: The objective of this study is to research the stability of glucose in samples collected into serum and plasma tubes, according to centrifuging following different waiting periods, and to identify which glucose tube would be most suitable for accurate glucose measurement in laboratories.

Material and Methods: A total of 12 venous blood samples were collected from volunteers subjected to OGTT (n=20) after fasting and after administering 75g of glucose. Serum (VACUETTE®CAT Serum Separator Clot Activator) and plasma (VACUETTE®FC Mix Tube) tubes were used. Centrifugation was performed as follows: in the 1st group, immediately at hour 0, group 2, and 3 after having been kept at room temperature for respectively 2 hours, and 4 hours.

Results: No significant changes were identified among the fasting and postprandial blood glucose measurements in the plasma tubes at 0, 2, and 4 hours ($p>0.05$). Significant changes were identified among the fasting and postprandial blood glucose measurements in the serum group at 0, 2, and 4 hours ($p=0.001$; $p<0.01$).

Discussion: Our study demonstrated that the plasma tube was most effective in preventing a clinically significant change in glucose concentration at room temperature.

Keywords

Glucose, Pre-Analytical Phase, Stability

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Introduction

Diabetes mellitus (DM) is a chronic and serious disease that occurs when the pancreas is unable to produce sufficient insulin or the body is unable to effectively use the insulin it produces, and it is characterized by high blood glucose levels [Available online at: https://www.who.int/publications/i/item/9789241565257]. Even though they may be below the diagnostic threshold for DM, blood glucose levels higher than optimal are an important source of mortality and morbidity [Available online at: https://www.who.int/publications/i/item/9789241565257]. A laboratory plasma glucose test is essential for diagnosing DM, impaired fasting glucose and/or impaired glucose tolerance, and especially for screening, and for diagnosing gestational DM in cases where HbA1c cannot be used [1,2]. On the other hand, there are studies using different sample types and different techniques for HbA1c measurements in various clinical and point care conditions [1,2]. Therefore, it is clear that plasma glucose measurement must be accurate and definitive for accurate patient classification according to international guidelines [1].

The analytic methods primarily used for glucose assessment are enzymatic analyses based on hexokinase (recommended) or glucose oxidase reaction [1]. These methods have been standardized with an inter-laboratory ambiguity (CV) <2.6% [2]. The diagnostic criterion for DM is ≥126 mg/dL fasting plasma glucose, which is a diagnostic point selected according to microvascular complications such as diabetic retinopathy [1].

The fact that phlebotomy units are nowadays located at a distance from central laboratories causes a delay of several hours in processing samples [3]. Since cellular metabolism is a process that continues even after phlebotomy, time and temperature conditions during the transfer of the samples become the most critical variables [3].

It is a known fact that glucose concentration decreases in samples kept in whole blood [4]. A 5-7% in vitro reduction per hour due to glycolysis has been reported in plasma glucose samples not centrifugated immediately [1]. In order to minimize in vitro glycolysis, the American Diabetes Association (ADA) and the National Academy of Clinical Biochemistry (NACB) recommend immediately placing an ice slurry in the sample tube, and separating the plasma from the cells in 30 minutes, and, if this is not possible, a sample tube containing a fast glycolysis inhibitor should be used [1].

Sodium fluoride/potassium oxalate (NaF/KOx) is the most widely found additive among glucose inhibitors. This tube ensures better glucose stabilization compared to conventional serum and lithium heparin tubes, but its effectiveness is inadequate [4]. The inhibiting effect of sodium fluoride (NaF) on glycolysis emerges only 2-3 hours after phlebotomy and causes a decrease in glucose concentration during this period [1].

Glucose concentration decreases >6% in NaF/KOx plasma samples kept at RT without having been centrifugated. After this period, the plasma glucose consumption rate drops, and at the end of a 4-hour period, the decrease in the glucose concentration reaches a maximum of 8%. Clinically significant hemolysis is seen in a maximum of 94% of the samples processed using the NaF/KOx additive [4].

The use of an FC Mix-citrate buffer tube instead of a tube containing NaF generates more stable and reliable results in the plasma glucose of samples kept at room temperature (RT) without centrifuging [4].

In recent years, the use of phlebotomy tubes containing NaF and citrate buffer have been recommended: acidification of blood to pH of <5.9 inhibits hexokinase and phosphofructokinase, which are enzymes that are involved in the upper stages of glycolysis, therefore causing a faster inhibition [1].

Glucose-specific plasma tubes containing NaF, citrate and EDTA were used in order to minimize glycolysis in our study. Our objective was to research the stability of the glucose in the samples collected into the serum and plasma tubes according to different centrifugation delays and to identify which tube would be most suitable for accurate estimation of glucose in a routine laboratory setting.

Material and Methods

The study was conducted with 20 volunteers subjected to an oral glucose tolerance test (OGTT) at the Xxxx Xxxx University, Xxxx Xxx Xxxx Xxxx Xxxx Hospital. Pregnant patients were excluded. A total of 12 venous blood samples per subject were collected. Six tube consisted of three serum and three plasma for 0. hour, 2nd hour, and 4th hour centrifugation in fasting and the same design were used for postprandial measurements. Namely, two batches of samples were taken after fasting, and after consuming a 75g glucose solution. 454243-VACUETTE® Tube 2.5 ml Z Serum Gel Clot Activator, 13x75 (Greiner Bio-One-Austria) tubes were used for serum, and 454513-VACUETTE® FC Mix 3 ml 13x75 (Greiner Bio-One-Austria) tubes were used for plasma. The serum tubes were turned upside-down 5-10 times, and the plasma tubes 10 times following phlebotomy. The collected samples were processed in 3 groups; the 1st group consisted of samples centrifugated immediately at hour 0. The 2nd group consisted of samples centrifugated after a delay of 2 hours. The 3rd group consisted of samples centrifugated after a delay of 4 hours (Table 1). Centrifugation was performed at 1800g for 10 minutes. The samples were analyzed using a Beckman Coulter AU 5821 instrument and glucose kit.

In OGTT patients, vascular access is established at hour 0. To ensure compliance with the optimum phlebotomy procedure at hour 0 and hour 2, phlebotomy was performed using a vascular access adapter (450210- VACUETTE® SAFELINK Holder with male luer-lock-Greiner Bio-One-Austria).

Ethics approval was obtained from the local committee (2020 / 532). Informed consent was obtained from all individuals included in this study.

Statistical Examinations

The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) software was used. When evaluating the study data, descriptive statistical methods were used. The compatibility of quantitative data with normal distribution was tested using the Shapiro-Wilk test. Repetitive measurements variance analysis was used in the intragroup comparison (IGC) of quantitative variables demonstrating a normal distribution, and the Bonferroni adjusted dual ratings were used in evaluating dual comparisons. The Friedman Test was used in the intragroup comparisons of quantitative variables that did not exhibit a

normal distribution, and the Bonferroni adjusted Wilcoxon signed-ranks test was used in evaluating dual comparisons. The Related groups t-test was used in the intragroup comparisons of quantitative variables demonstrating normal distribution. The Wilcoxon signed-ranks test was used in the intragroup comparisons of quantitative variables that did not demonstrate a normal distribution. Statistical significance was accepted as $p<0.05$.

Results

The results were evaluated by comparing blood glucose measurements performed in the plasma and serum tubes after fasting, and after the administration of glucose.

The mean 6.3±2.12, 13.36±3.67, 20.00±3.08 mg/dL differences found between the fasting plasma and serum measurements at hour 0, hour 2 and hour 4 were significant (p=0.001; p<0.01),

Table 1. The sample taking methodology for serum and plasma tubes

Tubes (n=20)		
$A1^0$, $A1^2$	$B1^0$, $B1^2$	Immediately after sampling, the $A1^0$, $A1^2$, $B1^0$, $B1^2$ tubes;
		- are turned upside-down 10 times
		- only the serum tube is kept for half an hour
		- centrifugated at 1800g for 10 min.
		- Glucose assessment is performed
$A2^0$, $A2^2$	$B2^0$, $A2^2$	The tubes are not subjected to centrifugation following phlebotomy;
		- Kept at room temperature for 2 hours
		- At the end of 2 hours, the tubes are centrifugated at 1800g for 10 min.
		- Glucose assessment is performed
$A3^0$, $A3^2$	$B3^0$, $A3^2$	The tubes are not subjected to centrifugation following phlebotomy;
		- Kept at room temperature for 4 hours
		- At the end of 4 hours, the tubes are centrifugated at 1800g for 10 min.
		- Glucose assessment is performed

A: CAT serum separator tube, B: Fc Mix plasma tube, 1, 2, 3: Indicates the centrifugation time, ^{0} : Indicates that the samples were taken for hour 0 in fasting, ^{2} : Indicates that the samples were taken for hour 2 following the administration of 75g glucose

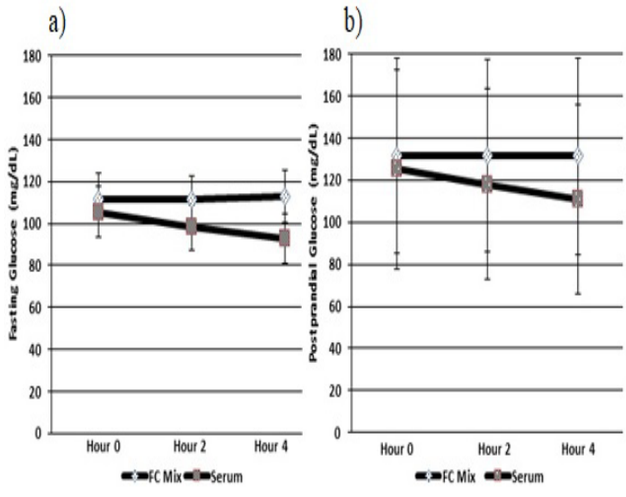

Figure 1. Distribution of FC Mix and Serum Sample Fasting (a) and Postprandial (b) Blood Glucose Measurements Over Time (mg/dL) (0h-4h)

respectively. No significant changes were identified among the fasting glucose measurements in the FC Mix group at 0, 2 and 4 hours (p>0.05). Significant changes were identified among the fasting glucose measurements in the serum group at 0, 2 and 4 hours (p=0.001; p<0.01) (Table 2). According to the results of dual comparison, the decrease in the mean of 7.45±3.76 mg/dL in fasting measurements at 2 hours compared to hour 0 was significant (p=0.001; p<0.01). The decrease in mean of 12.79±3.29 mg/dL in fasting measurements at 4 hours compared to hour 0 was also significant (p=0.001; p<0.01). The decrease in mean of 5.34±2.09 mg/dL in fasting measurements at 4 hours compared to hour 2 was significant as well (p=0.001; p<0.01) (Figure 1).

The difference in mean 6.63±3.67, 13.5±3.64, 20.65±3.58 mg/dL found between the postprandial plasma and serum measurements at hour 0, hour 2 and hour 4 was significant (p=0.001; p<0.01), respectively. No statistically significant changes were identified among the postprandial blood glucose measurements in the plasma group at 0, 2, and 4 hours (p>0.05). Significant findings were identified among the postprandial glucose measurements in the serum group at 0, 2 and 4 hours (p=0.001; p<0.01) (Table 2). According to the results of the dual comparison performed in order to identify the difference, the decrease in mean of 7.14±4.21 mg/dL in postprandial glucose measurements at 2 hours compared to hour 0 was significant (p=0.005; p<0.01). The decrease in mean of 14.51±5.72 mg/dL in postprandial measurements at 4 hours compared to hour 0 was also significant (p=0.001; p<0.01). Additionally, the decrease in mean of 7.38±3.13 mg/dL in postprandial measurements at 4 hours compared 2 hours was significant (p=0.005; p<0.01) (Figure 1).

Discussion

The citrate buffer/citric acid solution contained in the FC Mix plasma tube ensures pH-dependent enzyme inactivity, the EDTA acts as an anticoagulant, and NaF results in enzyme inhibition. Our study demonstrated that the plasma tube was most effective in preventing a clinically significant change in glucose concentration at RT.

This study provides useful data on the most suitable glycolysis inhibitor tube. The results have demonstrated that significant variation in glucose results is generated when the plasma tube is compared with serum in normal participants.

The serum data have demonstrated that the recommended 30-minute coagulation period is sufficient to allow important changes and that long-term contact with cells causes glucose consumption. In simple terms, this demonstrates that standard serum tubes are not suitable for accurate glucose estimation under normal laboratory processing conditions.

In the real world, samples in most laboratories are not processed and analyzed within 1 hour after being taken, but the serum is almost always used for the routine monitoring of glucose concentration and subsequent patient management.

The latest manuals on preanalytical and analytical conditions published by the ADA, the American Association of Clinical Chemistry (AACC, 2011), and the World Health Organisation (WHO, 2006) recommend the following: glycolysis must be

Table 2. Difference Between Fasting and Postprandial Plasma and Serum Sample Measurements Over Time

n=20 Fasting Measurements		Plasma (mg/dL)	Serum (mg/dL)	Difference	Test Value; p
Hour 0	Min-Max (Median)	91.7-134.5 (109.2)	82-125.2 (104.7)	6.37±2.12	t:13.424
	Mean±SD	111.85±12.22	105.48±12.02		[a]0.001**
Hour 2	Min-Max (Median)	93.9-128.3 (109)	81.1-112.3 (97.9)	13.36±3.67	t:16.26
	Mean±SD	111.39±11.49	98.03±10.96		[a]0.001**
Hour 4	Min-Max (Median)	91.1-132.7 (111)	72.2-108.9 (94.75)	19.99±3.08	t:29.003
	Mean±SD	112.68±12.41	92.69±11.81		[a]0.001**
Test Value		F:2.993	F:168.928		
p		[b]0.062	[b]0.001**		
Hours 0-2		1.000	0.001**		
Hours 0-4		0.245	0.001**		
Hours 2-4		0.115	0.001**		
Postprandial Measurements					
Hour 0	Min-Max (Median)	80.1-243.4 (116.6)	75.5-235.7 (110.1)	6.63±3.67	Z:-3.92
	Mean±SD	131.86±46.34	125.24±47.23		[c]0.001**
Hour 2	Min-Max (Median)	78.6-247.6 (114.7)	71.4-230 (100.4)	13.50±3.64	Z:-3.921
	Mean±SD	131.60±45.91	118.10±45.50		[c]0.001**
Hour 4	Min-Max (Median)	78.7-249.8 (115.4)	63.6-221.2 (93.8)	20.65±3.58	Z:-3.920
	Mean±SD	131.37±46.77	110.73±45.03		[c]0.001**
Test Value		x^2:0.9	x^2:40		
p		[d]0.638	[d]0.001**		
Hours 0-2		1.000	0.005**		
Hours 0-4		1.000	0.001**		
Hours 2-4		1.000	0.005**		

[a]Paired Samples Test, [b]Repeated Measures Test, **p<0.01, [c]Wilcoxon Signed Ranks Test, [d]Friedman Test, **p<0.01

minimized by immediate separation of the plasma from cells by centrifugation, or by placing the tubes on ice immediately after phlebotomy, and performing centrifugation within 30 minutes [5].

Since applying either method is logistically difficult in everyday practice, it is a widespread practice to add NaF to the blood collection tube as a glycolysis inhibitor [5]. However, the inhibitory effect of NaF on glycolysis emerges only 2-3 hours after phlebotomy resulting in a decrease in glucose concentration during this period [1]. Therefore, the use of NaF alone as a glycolysis inhibitor is considered inadequate by the AACC and WHO [6].

The combination of different anticoagulants in plasma tubes offers an important advantage in the preservation of glucose compared to tubes containing only serum, heparin, EDTA, NaF/KOx or citrate.

It is clear that the prevention of glycolysis would give rise to a more accurate diagnosis and timely management of patients, which, in turn, may be useful, reducing risks for patients and the healthcare system; in the same manner, it will render results more reliable during lengthy storage periods before the analysis is completed.

ADA and NACB manuals recommend that plasma glucose analyses must be performed in tubes that are treated according to the gold standard for handling blood samples, or in collection tubes that contain a fast-acting glycolysis inhibitor such as a citrate buffer [6].

Serum, lithium-heparin or NaF/EDTA tubes, used on their own, miss the analytical target for the allowed deviation. This way, plasma tubes allow accurate identification of glucose, which is among the fundamental criteria for diagnosing DM.

Citrate buffer generates samples with a lower rate of hemolysis compared to NaF. It has been demonstrated that the glucose concentration measured in samples containing citrate buffer yields more stable and accurate results compared to the glucose value measured in samples containing other additives. The hexokinase enzyme in the plasma of hemolyzed samples erroneously decreases the glucose concentration measured using the reference hexokinase measurement method [4].

The evaluation of the plasma glucose in the samples measured in the tubes containing citrate buffer reflects the real (in vivo) glucose concentration more reliably. These innovations in additives give rise to important questions relating to the existing glucose reference ranges for diagnosis, which have been identified and verified using other additives [4].

The literature emphasizes that the transition from tubes containing NaF to tubes containing a citrate buffer causes a marked increase in the existing value ranges of glucose measurements for the diagnosis of DM. This increase necessitates further research to identify whether it is necessary to re-evaluate the reference ranges and the threshold values for diagnosis [4].

The tube we have used is a special blood collection tube containing a combination of EDTA anticoagulant, NaF, and citrate. The VACUETTE® FC Mix Tube, contains additives in dry/powder form.

The use of the glycolysis inhibitor in granular form offers a pre-analytic advantage by eliminating any dilution effect that may

occur as a result of insufficient tube filling when using a liquid additive [7].

In short, when a gel barrier separates the serum from the cells, glucose is very stable; however, the separation of the serum from the cells in 30 minutes is not a practical solution to the glycolysis problem in practice, since the period of time expiring between collection and centrifugation is variable and uncontrolled.

Conclusion

The objective of this study was to identify the most suitable tube for accurate glucose estimation in a routine laboratory under identical conditions. In summary, the use of an plasma tube is useful for obtaining more accurate results and a reliable glucose concentration, higher stability in glucose levels that otherwise change in time, samples with less hemolysis compared to NaF tubes, and to prevent the risk of misdiagnosis in DM based on false low glucose results stemming from uncentrifuged samples [4].

Since the combination of additives in the plasma tube offers an important advantage in the protection of glucose levels compared to the serum tube, plasma tube is the most suitable tube to minimize glycolysis. It prevents false-negative findings in diagnosing DM due to its characteristics of preventing glucose loss and maintaining stability through long waiting periods prior to analysis. Our results demonstrated that the plasma tube was most effective in preventing a clinically significant change in glucose concentration at room temperature.

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Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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